REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE January 1995	3. REPORT TYPE AND DATES COVERED Letter Report January 1995

4. TITLE AND SUBTITLE
Preliminary Coupled Diffusion/Reaction Model for the Bioremediation of TNT Through the Use of Plant Enzymes

5. FUNDING NUMBERS
N/A

6. AUTHOR(S)
L.J. Criscenti

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Battelle Pacific Northwest Laboratory
Richland, WA 99352

8. PERFORMING ORGANIZATION REPORT NUMBER
N/A

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
SERDP
901 North Stuart St. Suite 303
Arlington, VA 22203

10. SPONSORING / MONITORING AGENCY REPORT NUMBER
N/A

11. SUPPLEMENTARY NOTES
Letter report prepared for the U.S. EPA Environmental Research Laboratory. No copyright is asserted in the United States under Title 17, U.S. code. The U.S. Government has a royalty-free license to exercise all rights under the copyright claimed herein for Government purposes. All other rights are reserved by the copyright owner.

12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release: distribution is unlimited	12b. DISTRIBUTION CODE A

13. ABSTRACT *(Maximum 200 Words)*

Research at the Environmental Research Laboratory-Athens (ERL-Athens) on environmental transformations of nitroaromatic compounds has led to the isolation and characterization of a reductive protein from sediments and aquatic plants that readily reduces nitroaromatic compounds to the corresponding amino compounds. Under aerobic conditions, these nitroaromatic amines are readily oxidized to the catechols.

The protein was isolated from sediments by extraction and purified by column chromatography using TNT reduction as an essay for protein activity. The purified protein was used to obtain the monoclonal antibody later used to determine the presence of this protein in solution. A field immunoassay was subsequently developed to help locate the source of protein.

14. SUBJECT TERMS SERDP, chromotography, nitroaromatic compounds, catechols, monoclonal antibody, immunoassay	15. NUMBER OF PAGES 37
	16. PRICE CODE N/A

17. SECURITY CLASSIFICATION OF REPORT unclass	18. SECURITY CLASSIFICATION OF THIS PAGE unclass	19. SECURITY CLASSIFICATION OF ABSTRACT unclass	20. LIMITATION OF ABSTRACT UL

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

PRELIMINARY COUPLED DIFFUSION/REACTION MODEL
FOR THE BIOREMEDIATION OF TNT THROUGH THE USE OF PLANT ENZYMES

L. J. Criscenti
Battelle Pacific Northwest Laboratory
Richland, WA 99352

Letter Report
for the
U. S. EPA Environmental Research Laboratory
Athens, Georgia 30605

January 1995

INTRODUCTION

Research at the Environmental Research Laboratory-Athens (ERL-Athens) on environmental transformations of nitroaromatic compounds has led to the isolation and characterization of a reductive protein from sediments and aquatic plants that readily reduces nitroaromatic compounds to the corresponding amino compounds. Under aerobic conditions, these nitroaromatic amines are readily oxidized to the catechols.

The protein was isolated from sediments by extraction and purified by column chromatography using TNT reduction as an assay for protein activity. The purified protein was used to obtain the monoclonal antibody later used to determine the presence of this protein in solution. A field immunoassay was subsequently developed to help locate the source of protein.

Field sampling and analysis indicated that the aquatic plant, stonewort, had high activity and the nitrate reductase protein complexes were present. Studies indicated that stonewort had a large capacity to reduce TNT to triamino-toluene (TAT). Breakdown products of TAT were detected by capillary electrophoresis.

Six TNT-contaminated soil samples were treated with the nitrate reductase protein isolated from pond sediment resulting in reduced amino compounds, and a second set of six samples were treated using the stonewort plant as a source of the protein. The isolated nitrate reductase protein had the potential to remediate soils contaminated with low concentrations of TNT, and stonewort had the potential to remediate soils contaminated with high concentrations of TNT. (Wolfe et al. in prep).

Because of the discovery that the protein produced by stonewort has the potential to breakdown TNT, ERL-Athens is

2

conducting research to develop sufficient understanding of the plant/TNT system to design a demonstration of the use of plants to remediate TNT in the field. The objectives of the research are to (1) develop realistic models of TNT transport and biodegradation to assist in the design of larger-scale laboratory and field experiments; and (2) ensure collection of the data required to extrapolate from laboratory observations to field conditions through the use of a model.

Two remedial scenarios are being considered. In the first scenario, a basin is filled with TNT-contaminated soil and water, plants are introduced to the surface water above the soil, and solute transport between the soil and surface water is strictly by diffusion. In the second scenario, the basin is again filled with TNT-contaminated soil and water, the plants are introduced, and pumping is used to ensure the continuous flow of water through the soil column, thereby increasing the rate of contact between TNT and the plant enzymes. Other scenarios have also been proposed: for example, mixing the plant or the plant enzyme into the topsoil has been suggested.

The objectives of this report are to (1) review the data for soil contamination by TNT, and the chemical characteristics and behavior of TNT in the environment, (2) describe the development of a 1-D diffusion/reaction model that mimics the diffusion-only remedial scenario described above, and (3) compare the model calculations to experimental observations.

DATA SUMMARY AND REQUIREMENTS

The rate-limiting step in the breakdown of TNT by a plant- or moss-excreted enzyme is expected to be the rate at which TNT migrates through the soil. Migration of TNT through the soil is determined by (1) TNT diffusion through the soil porewater, (2) TNT

3

adsorption and desorption, and (3) TNT dissolution. Thus, the behavior of TNT is influenced by the properties of the soil and those of TNT. The following data may be used to bound input parameter values for a TNT migration model. Because the field demonstration is expected to occur at the Alabama Army Ammunition Plant (AAAP), Childersberg, Alabama, the information available on soils from this site is described below.

SOIL CHARACTERISTICS

According to the AAAP Feasibility Study (ESE 1986), contamination at the AAAP falls into five categories: (1) explosive residue -- weathered residues of 2,4,6-TNT and tetryl, (2) nitroaromatics contamination -- environmental media containing nonreactive concentrations of explosives, (3) asbestos, (4) lead, and (5) numerous 55-gallon drums dumped into an old well. Explosive residue (> 10 to 20% TNT by weight) is present in the 13,600 square yards of the Red Water Ditch Spoil Banks, where numerous discrete pieces of TNT (average diameter 0.5 in.) are scattered on the ground surface. Twenty-five percent of the industrial sewer lines are also contaminated with explosives. Nitroaromatic contamination constitutes most of the contamination at the site and is present in soils, sediments, building surfaces, surface water, and groundwater.

The maximum TNT concentrations detected in surveys (ESE 1986) are listed in Table 1. The Preliminary Pollutant Limit Value (PPLV) methodology represents an approach for establishing criteria for remediation based on site-specific exposure and risk assessment techniques and is documented by Small (1984). The PPLV or maximum allowable residual concentrations of TNT for soil set by the methodology is most stringent for subsistence farming and residential housing scenarios. The PPLV for TNT is 1.3 ppm if mutagenic effects are not considered and 0.013 ppm if they are.

4

Eight samples of soil from the AAAP were analyzed at ERL-Athens (Wilhelm, 1991). TNT concentrations in these samples are reported in Table 2. Wilhelm (1991) also reported the results of inductively coupled plasma (ICP) analysis of the samples and particle size distribution data.

The Childersberg soil consists primarily of clay, with some silt, sand, and occasional chert boulders. It ranges in thickness from 3 to 30 ft (S. McCutcheon, Research Environmental Engineer, U.S. Environmental Protection Agency, pers. comm. 1995). No depth profiles of TNT concentrations in the soil at AAAP are available. However, at the Umatilla field site, another TNT-contaminated Army ammunition plant, TNT has been found to depths of 20 ft (S. McCutcheon, Research Environmental Engineer, U.S. Environmental Protection Agency, pers. comm. 1995), with maximum TNT concentrations observed in the top 3 to 5 inches.

To model bioremediation scenarios effectively for the AAAP site, the bulk density and porosity for the soil should be measured. In addition, data on the percentages of clay minerals and Fe oxides present in the soil and a measure of the cation exchange capacity (CEC) of the soil would be useful. Depth profiles of TNT concentrations in the soil at AAAP would provide site-specific information for the initial conditions imposed in the TNT-migration model.

PROPERTIES OF TNT

Solubility

The solubility of TNT in water is 130 ppm or 0.013 g per 100 g water at 20°C (Spanggord et al. 1980a).

Rate of Dissolution

The dissolution rate of TNT is not expected to be significant relative to the rate of TNT diffusion through soil. However, the rate of dissolution is not fully known; it will depend on both the form of TNT (crystalline or amorphous) and the size and surface area of the TNT particles.

TNT nuggets are found in the first few inches of surface soil at several army ammunition plant sites. These nuggets range in size from approximately 0.2 cm^3 to 7 cm^3. No mineralogical analysis of the nuggets has been performed. Because the nuggets are found close to the soil surface, they may be coated with weathering products that are less soluble than pure TNT, slowing TNT dissolution.

Experiments were performed at Athens-ERL to determine the dissolution rates of TNT nuggets found at the AAAP field site. Four nuggets (A, B, C, and D) of increasing size were each placed in 1 L of water (Table 3). Then TNT concentrations in solution were measured over 21 days. TNT concentrations through time are plotted in Figure 1. The TNT nuggets were irregular in shape; the volumes and surface areas reported in Table 3 were estimated by assuming that the TNT nuggets were rectangular parallelograms.

In general, the larger the TNT nugget, the more dissolved TNT was found in solution at any given time. However, nugget C dissolved more slowly than nugget B, suggesting that there may be differences in surface coatings or mineral form among TNT nuggets.

Assuming a linear kinetic rate law (C = kt, where C is concentration, k is a rate constant, and t is time), rate constants between 1.2 x 10^{-4} and 1.9 x 10^{-3} mg/L/min were calculated for the four experiments. The linear kinetic rate law adequately

describes the dissolution of nuggets B and D (R^2 =0.98, 0.93), which dissolve more quickly than nuggets A and C (R^2 = 0.60, 0.74).

Adsorption

It is frequently assumed that the adsorption of organic compounds is predominantly a function of the percent of organic matter in the soil. The log K_{ow} for TNT is 2.592, where K_{ow} is defined as the concentration of TNT in the octanol phase divided by the concentration of TNT in the aqueous phase. The log K_{oc}, where

$$K_{oc} = (\mu g \text{ adsorbed/g organic carbon})/(\mu g/mL \text{ solution}) \qquad (1)$$

can be estimated from the K_{ow} or the solubility, S. Using regression equations provided by Lyman et al. (1982), log K_{oc} values ranging from 2.213 to 2.80 were calculated for TNT. If there is no clear match between the chemical classes used to derive the regression equations and the chemical class of the organic compound of interest (in this case, TNT), the following two equations are recommended for use:

$$\log K_{oc} = -0.55 \log(S) + 3.64 \qquad (2)$$
$$\log K_{oc} = 0.544 (\log K_{ow}) + 1.377 \qquad (3)$$

These equations yield log K_{oc} values for TNT of 2.8 and 2.79 respectively, suggesting that the high end of the calculated range (2.213 to 2.80) is more appropriate for TNT.

Few adsorption and desorption experiments for TNT in soils have been performed. Spanggord et al. (1980b) conducted five experiments and determined an average K_d of 53 ± 20 and an average K_{oc} of 1600 ± 600 (average log K_{oc} = 3.2) for sediment from the Holston River. The K_d was calculated as the amount of solute adsorbed per unit mass of solid, (C_s), divided by the equilibrium

concentration of the solution at a constant temperature, (C_w):

$$K_d = C_s/C_w \tag{4}$$

Several studies have shown that this equation describes sorption of of neutral organics on natural sediments from dilute solutions (Smith et al. 1977, Karickhoff et al. 1979). Because neutral organic species are primarily sorbed into the organic films of sediments, adsorption coefficients are often normalized for the fraction of organic carbon present (F_{oc}) in the sediment (Karickhoff et al. 1979). Then,

$$K_{oc} = K_d/F_{oc} \tag{5}$$

This linear relationship between K_d and F_{oc} implies that F_{oc} is independent of the density of available adsorption sites on the organic matter, and adsorption on the clay fraction of the sediment is small. For the Holston River sediment, F_{oc} was measured by Spanggord et al. (1980b) as 0.033.

Pennington and Patrick (1990) performed TNT adsorption and desorption studies using soil samples from 13 U.S. Army ammunition plants. Steady-state adsorption and desorption of TNT with test soils was reached within 2 hours. [Steady state was observed by Spanggord et al. (1980b) only after 140 hours.] Batch TNT adsorption isotherms were best-fit by the Langmuir adsorption model and had an average K_d of 4.0 for all soils. The TNT K_d values were most correlated with extractable Fe, CEC, and clay content (where "clay" refers to particle size, not mineralogy). Almost all of the adsorbed TNT had been desorbed after only three sequential desorption cycles. These results suggest that adsorption in soils will not greatly retard the movement of TNT. Adsorption and desorption were not pH dependent; however, oxidized conditions consistently decreased adsorption of TNT compared to reduced

8

conditions.

Ainsworth et al. (1993) conducted batch and column experiments on soils from U. S. Army ammunition plants to develop adsorption models for TNT under abiotic conditions. The batch experiments demonstrated that sorption data were best-fit with the Freundlich model:

$$q = K_f C^{1/n} \tag{6}$$

where q is the solid phase concentration of contaminant (μg/g), K_f is the adsorption coefficient, C is the equilibrium solution concentration (μg/mL), and n is the Freundlich characteristic constant. Like Pennington and Patrick (1990), Ainsworth et al. (1993) concluded that the sorption of TNT is not solely the result of hydrophobic partitioning to the organic carbon (OC) phase of the soils, but rather due in part to sorption onto inorganic solids. The parameters K_f and n are best fit by the following equations:

$$K_f = -7.69 + 1.6(pH) + 0.35(CEC) \tag{7}$$

$$1/n = 0.56 + 0.15(OC). \tag{8}$$

This model is statistical in nature, lacking a mechanistic basis at this time.

The soil in column experiments conducted by Ainsworth et al. (1993) exhibited reversible TNT sorption, but some mass loss occurred for all soils. When in contact with the soil, TNT was observed to undergo a reduction of one or more NO_2^- groups to NH_2^- groups. The slow step in this process appeared to be diffusion through micropores, since more TNT mass loss occurred at lower porewater velocities in the column experiments. It appeared that sorption was required before TNT could transform. The soil

constituents that acted as electron donors for this reaction are unknown; one hypothesis is that the electron donors in the soil are Fe sulfides.

Rates of Diffusion

Information on how to calculate the diffusion coefficient of TNT in water, D, for organics, was obtained from J. Szecsody, Senior Research Scientist, Pacific Northwest Laboratory (pers. comm. 1993). For salt tracers, D is approximately 2×10^{-5} cm^2/sec, and it is slightly smaller for TNT.

According to Freeze and Cherry (1979),

$$C_i(x,t) = C_o \; erfc(x/2 \; (D^*t)^{0.5}) \tag{9}$$

where C_o is the concentration of solute i at some initial time, $C_i(x,t)$ is the concentration of species i in the x direction over time t, and D^* is the apparent diffusion coefficient for nonadsorbed species in porous media. Values of 1×10^{-10} and 1×10^{-11} m^2/sec for D^* are typical for nonreactive chemical species in clayey geologic deposits. Values for coarse-grained unconsolidated materials can be as high as 2×10^{-9} m^2/sec. At a rate of 1×10^{-10} m^2/sec, 0.01 of the initial concentration will have traveled approximately 3 m in 100 years.

Major inorganic ions in groundwater have diffusion coefficients (D) in the range of 1×10^{-9} to 2×10^{-9} m^2/sec at 25°C. The coefficient of molecular diffusion for a solute in a porous medium, D^*, is calculated as

$$D^* = \omega D \tag{10}$$

where ω, the tortuosity factor, is less than 1 and takes into

account the effect of the solid phase of the porous medium on diffusion. A typical tortuosity factor is 0.66 (Freeze and Cherry 1979).

Soil samples have been treated with an enzyme isolated from a pond sediment and with the stonewort plant. In the experiments with stonewort, the breakdown of TNT was

$$TNT + e^- \xrightarrow{\text{enzyme}} TNT\ Byproducts \tag{11}$$

where e^- is the electrons provided by the plant or other materials present, and the enzyme acts as a catalyst in the reaction. The TNT breakdown pathways are illustrated in Figure 2.

The source of electrons in the experiments with the isolated enzyme is unknown. The reaction was observed to slow down and the change in reaction rate may be due to the lack of electrons, the exchange of Fe with Pb or Zn on the Fe-S enzyme, or bacterial destruction of the enzyme (L. Carreira, Principal Investigator, Technology Applications, Inc., pers. comm. 1993).

The enzyme appears to be intracellular and extracellular in nature. However, reactions between the enzyme and TNT appear to occur outside of the stonewort plant. Transport of the enzyme away from the plants in the surface water or into the soil has not been measured, and the mechanism of reaction between the enzyme and the TNT has not been fully characterized. The stoichiometry of the reaction between the TNT and the enzyme has not been determined.

The half-life of TNT is dependent on the concentration of TNT present in solution. Experiments performed in air with the enzyme present in sediment indicate that at low concentrations of TNT [1×10^{-6} M (0.23 mg/L) to 1×10^{-5} M (2.27 mg/L)] the half-life of TNT is between 50 and 150 minutes. The half-life for TNT present in solution with the enzyme is 20 minutes for a concentration of

1.5 x 10^{-6} M (0.34 mg/L) and 40 minutes for a concentration of 3 x 10^{-6} M (0.68 mg/L). The half-life of TNT with the plant present is 70 minutes. Patrick, a graduate student at University of Georgia, has determined that under reduced conditions with cysteine present, the half-life of TNT is approximately 1 hour even for initially high TNT concentrations (L. Carreira, Principal Investigator, Technology Applications Inc., pers. comm. 1993).

MODEL FOR THE DIFFUSION-ONLY SCENARIO

The first field demonstration under consideration is to place contaminated soil in a lined basin, flood the basin, import plants to grow in the surface water, and then wait for TNT to diffuse through the soil column and contact the plants near the surface. The enzymes produced by the plants would then proceed to break down the TNT.

To mimic this scenario, a simple two-step, one-dimensional transport model was developed. This model represents a one-dimensional vertical soil column of a flooded basin (Figure 3). Diffusion of TNT out of the soil column into the surface water is simulated; however, no TNT is allowed to escape the lower boundary of the soil column. Because the reaction between TNT and the enzyme has been observed to be rapid, and the enzyme is assumed to be ubiquitous in certain surface water/plant environments, the upper boundary of the column is treated as a zero-concentration boundary. Diffusion of TNT through the soil is assumed to be the rate-limiting step in TNT breakdown.

The soil column is divided into layers. Solid, adsorbed, and dissolved TNT may be initially present in each layer. TNT diffusion is allowed for one time step; then mass transfer between solid, adsorbed, and dissolved TNT allows reequilibration of the

system before the next time step. TNT diffusion from each layer to the others in the column is described by the probability that mass from one layer will travel to another in one time step. Mass transfer between solid and dissolved TNT is controlled by TNT solubility. Mass transfer between adsorbed and dissolved TNT is governed by a linear K_d.

A direct simulation method, the Markov method, was chosen to calculate solute transport in the model. The user specifies the initial conditions in the soil column and the geochemical reactions that are allowed to occur during the simulation. A two-step coupling algorithm combines the transport and geochemical processes in the code. During each time step in the simulation, TNT is distributed along the one-dimensional soil column according to the diffusion properties of TNT in a porous media. Then the TNT in solution in each layer of the soil column reacts with the adsorbed and solid phase TNT already present in that layer. This coupled two-step Markov approach to solving solute transport problems is similar to those described by Hostetler and Erikson (1989), Criscenti et al. (1990), and Schramke et al. (1992).

The mass of TNT present in the aqueous solution in each layer is stored as a time-dependent state vector. For a steady-state flow system, the state vector at time t+1 depends only on the state vector of the chemical component at the previous time, t. The transition from one time step to the next is represented by a linear operator, the Markov diffusion matrix. Each entry in the diffusion matrix represents the fraction of mass in layer i at time t+1 that was transported from layer j in the previous time step (t). The state vector at time step t+1 (S_{t+1}) is obtained by multiplying the state vector at the time step t (S_t) by the diffusion matrix:

14

$$S_{t+1} = [T] S_t \qquad (12)$$

DIFFUSION EQUATIONS

Diffusion from one layer into the neighboring layers is calculated according to the equation for diffusion from a point source. A seven-layer probability or diffusion vector is used. The origin is assumed to be in the middle of the vector and the probability of staying in the center layer and the probabilities of entering the surrounding layers are calculated. For each time step, the diffusion vector is effectively placed over each layer in the soil column and the mass of TNT remaining in solution in the layer after diffusion from that layer is added to the sum of the mass of TNT entering that layer from the surrounding layers (Figure 4). The mass that leaves the soil column and enters the surface water is assumed to be removed from the system through breakdown reactions with the plant enzyme.

The equation to calculate diffusion from a point source is

$$p = \frac{1}{2\sqrt{\pi D^* t}} e^{\frac{-x^2}{4D^* t}} \qquad (13)$$

where D^* is the apparent molecular diffusion coefficient, t is the time step, and x is the distance from the origin.

This function is integrated over each layer thickness to create the diffusion vector. The probability mass in a region bounded by $x_a \leq x \leq x_b$ is

$$P = \int_{x_a}^{x_b} \frac{1}{2\sqrt{\pi D^* t}} e^{-\frac{x^2}{4D^* t}} \qquad (14)$$

16

The solution to Equation 14 is

$$P=0.5\times[erfc(\frac{X_a}{\sqrt{4D^*t}})-erfc(\frac{X_b}{\sqrt{4D^*t}})]$$ (15)

TNT CHEMISTRY

The total mass of TNT present in each layer is entered and distributed between aqueous, adsorbed, and solid phases on the basis of a linear K_d and the solubility of TNT. If adsorption of TNT is considered, then the mass of TNT is divided into adsorbed and aqueous masses before the precipitation or dissolution of TNT solid is considered.

A first-order kinetic rate law for the breakdown of TNT by the plant enzyme is also included in the model. This algorithm allows for the breakdown of TNT in the soil porewater based on the assumption that the enzyme is present in abundance throughout the porewater.

The kinetics of TNT breakdown is considered using the aqueous TNT concentrations in each layer after transport by diffusion has changed the layer concentrations and before geochemical equilibrium is established through TNT adsorption and precipitation/ dissolution.

MODEL APPLICATION

Several test calculations designed to be similar to batch experiments were performed with the coupled diffusion/reaction model. The batch experiments consisted of a 2-cm layer of sediment

17

covered by surface water with the stonewort plant. The soil sample SO-1, which contains 2065 μg TNT per gram of soil, was used as the starting material. It was assumed that TNT is evenly distributed throughout the column and that TNT can be both adsorbed and present as a discrete solid phase.

The following parameters were used to calculate the migration of TNT in the soil in the beakers that were simulated: (1) soil porosity of 0.5, (2) soil bulk density of 1.325 g/cm^3, (3) soil column length of 0.02 m, (4) an approximate molecular diffusion coefficient (D) of 1.0×10^{-5} cm^2/sec (J. Szecsody, Senior Research Scientist, Pacific Northwest Laboratory 1993), (5) a tortuosity factor (ω) of 0.66 (Freeze and Cherry 1979), and (6) an apparent diffusion coefficient ($D^* = \omega D$) of 6.6×10^{-6} cm^2/sec.

The hypothetical soil column used in the calculations is depicted in Figure 3. TNT diffuses into the surface water where it breaks down. The point of contact between the sediment and overlying surface water is defined as 0.0 m and the base of the soil column is 0.02 m.

For Case 1, it is assumed that no solid or adsorbed TNT is present in the soil column and the aqueous concentration of TNT is equal to the solubility of TNT [5.7236×10^{-4} M (130 mg/L)]. The resulting concentration of TNT versus time is plotted in Figure 5. The dissolved TNT diffuses out of the 2-cm thick soil layer within 15 days.

For Case 2, the mass of TNT present in the soil, estimated for sample SO-1 as 2065 μg/g, is distributed between a discrete solid phase and the porewater. TNT precipitation and dissolution are considered. The calculated aqueous concentration of TNT and the mass of solid TNT present through time at different depths in the soil column are illustrated in Figure 6, parts A and B,

respectively. Experiments in the laboratory on other soil samples (Wolfe et al. in prep) suggest that the calculated diffusion of TNT from the soil column is slower than that observed in the laboratory. In the experiments, the concentration of TNT in supernatants and sediments decreased significantly within 45 days when stonewort plants were present in the water column above. As shown in Figure 6B, the model calculations indicate that even if the stonewort maintained a zero TNT concentration in the surface water, solid TNT would still be present in the lower half of the 2-cm soil column after 90 days. The difference between the calculated and observed diffusion rates for TNT may be caused by limited mixing of sediment with overlying surface water during sampling. Such limited mixing would cause advection and introduce the enzyme into the sediment.

For Case 3, TNT diffusion and adsorption in the soil column are considered. A K_d of 4 is used (Pennington and Patrick 1990). To eliminate the possibility of TNT precipitation in this simulation, the mass of TNT in the soil column is selected to be less than the mass of TNT in sample SO-1.

In Case 4, the three processes, diffusion, adsorption and precipitation or dissolution, that influence the rate of TNT removal from the soil column are considered. The simulated TNT concentration in Case 4 is taken to be 2065 μg/g. Figures 7 and 8 illustrate the calculated porewater concentrations of TNT at various positions within the soil column for Cases 3 and 4, respectively. Adsorption retards the diffusion of TNT in the soil column. A comparison of Case 2 with Case 4 suggests that adsorption in combination with precipitation retards the removal of TNT from the soil column more that precipitation alone.

Calculations were also performed to examine Case 5 in which the plant enzyme is present throughout the soil column.

19

Experimental results suggest that in the presence of the enzyme, the half-life of TNT is as little as 20 minutes (Wolfe et al. in prep). Based on this estimate, the TNT present in a 2-cm deep layer of soil would disappear in two days (Case 5). Although the assumption that the enzyme is present throughout the soil column grossly overestimates the ability of the enzyme to diffuse into the sediment, it provides an estimate of the maximum rate at which TNT might disappear from a soil column. This case might be compared to a field demonstration in which the plant enzyme or plant is mixed with the top soil, eliminating the need for TNT to diffuse to the plant before TNT breakdown begins.

These calculations bound the experimental results. Cases 2 and 4 demonstrate that diffusion alone cannot account for the disappearance of TNT from a 2 cm column of soil within 90 days. Case 5 demonstrates that the presence of the enzyme throughout the soil column would result in the disappearance of TNT within 2 days. The experiments (Wolfe et al. in prep) indicate that the TNT will disappear within 45 days. Several parameters in the experiments conducted so far were poorly controlled, e.g., variations in TNT concentrations in the soil samples due to the small quantity of soil used in each experiment, and diffusion of the plant enzyme into the sediment.

COMPARISON OF NUMERICAL MODEL WITH ANALYTICAL SOLUTION

For a specific case, results of calculations with the diffusion algorithm in the numerical model are compared to the analytical solution provided by Ogata (1970) for one-dimensional, steady-state advective-diffusion. The analytical solution is

$$C = C_1 + 0.5(C_0 - C_1) \, erfc(\frac{x - \frac{v}{por*t}}{2\sqrt{D^*t}}) + e^{(\frac{xv}{D^*por})} \, erfc \frac{(x + \frac{v}{por*t})}{2\sqrt{D^*t}} \quad (16)$$

where C_0 and C_1 are the boundary conditions, v is velocity, D^* is the apparent diffusion coefficient, x is depth, and por is the porosity. To solve a diffusion-only case, the velocity is set to zero. The porosity may be arbitrarily set to 1, because once the velocity is set to zero, eliminating the effects of advection/dispersion from the problem, all terms in the equation that include the porosity are also zero. C_0 and C_1 are set to 1 and 0, respectively. This reduces Equation 16 to

$$C = 1 + 0.5\, erfc\left(\frac{x}{2\sqrt{D^*t}}\right) \qquad (17)$$

The results of the analytical solution for D^* of 2 x 10^{-5} cm^2/sec and a depth of 50 cm are illustrated in Figure 9A.

The problem defined by Ogata (1970) is different from the TNT diffusion problem discussed in this report in two ways. First, in the problem defined by Ogata (1970), the initial conditions include a high concentration of contaminant at one end of the soil column and no contamination throughout the rest of the soil column. In the TNT diffusion problem, TNT is assumed to be homogeneously distributed throughout the soil. The problem solved by Ogata (1970) does not include loss of contaminant from the soil column to an overlying water column as in the TNT diffusion problem.

To compare the numerical model described in this report to Ogata's (1970) analytical solution, the computer code was modified to allow different initial concentrations of TNT in different segments of the hypothetical soil column and to eliminate mass transfer out of the soil column. These modifications do not alter the diffusion algorithm itself. A 50-cm column was divided into 250 layers and the initial conditions included a large mass of TNT in one layer at the base of the hypothetical soil column, and none in the rest of the column. Figure 9B illustrates that, with a

diffusion coefficient of 2 x 10^{-5} cm^2/sec, a porosity of 1, layer thicknesses of 0.2 cm, and time steps of 0.01 days, the calculated concentration with distance profiles closely matched those of the analytical solution for periods of 5, 10, and 60 days. Both the layer thickness and time step size are used in the calculation of the diffusion; for a successful calculation, both must be selected so that over 95% of the mass is accounted for in the seven-layer probability vector.

CONCLUSIONS/FUTURE WORK

A second suite of experiments was conducted using soil sample SO-2, which contains 6000 μg of TNT per gram of soil. Soil was placed in four 1-L beakers; the depth of soil in the beakers ranged from 0.5 cm to 2 cm. Eight hundred milliliters of water were added to each beaker. The standing water above the soil was monitored until the water was found to be saturated with TNT. Stonewort was added to the beakers once the surface water was saturated with TNT. Within several days, no TNT remained in the surface water.

Continued diffusion of TNT out of the soil and TNT breakdown by the plant enzymes was inferred from the continued observation of TNT breakdown products in the surface water over a period of six weeks. No increase in the concentration of the breakdown products of TNT (dinitromonoaminotoluene, mononitrodiaminotoluene, and triaminotoluene) was observed, because these compounds degrade more quickly than TNT in the presence of the plant enzymes.

The model described in this report calculates diffusion, solubility, adsorption, and breakdown of TNT within the soil column. A mass balance is performed, tracking the mass of TNT that leaves the soil column through time. Because the model was designed so that (1) the plant enzyme is ubiquitous in the surface

water column and (2) diffusion of TNT through the soil column is the rate-limiting step, the model assumes a zero concentration boundary at the surface of the soil column.

To completely describe the second suite of experiments, this model may need to be modified to include diffusion of TNT within both the soil column and the surface water. This modification would allow a comparison between the observed rates with which the surface water reaches the TNT solubility limit and the calculated rate of TNT entering the surface water. The diffusion of the enzyme from the floating plant into the surface water and sediment may also have to be considered.

Because the abundance of TNT breakdown products is easily monitored in the surface water, it would also be useful to develop an algorithm that kinetically describes the sequence of TNT and byproduct breakdown reactions using their corresponding half-lives.

Additional enhancements to the coupled diffusion/reaction model might include (1) incorporating the empirical adsorption model developed by Ainsworth et al. (1993) and (2) developing a probabilistic approach to describe the kinetics of TNT breakdown. The first enhancement would allow for a comparison of the TNT adsorption properties of different soil types. The second enhancement would lead to a method of combining the kinetic and transport time steps.

REFERENCES

Ainsworth, C. C., S. D. Harvey, J. E. Szecsody, M. A. Simmons, V. I. Cullinan, C. T. Resch, G. H. Mong. 1993. Relationship between the Leachability Characteristics of Unique Energetic Compounds and Soil Properties. Pacific Northwest Laboratory, 120 pp.

Criscenti, L. J., C. J. Hostetler, and R. J. Serne. 1990. A Model for Cement Leaching: Diffusion and Mass Transfer. American Ceramic Society, 92nd Annual Meeting Abstracts, p. 352.

Environmental Science and Engineering, Inc (ESE). 1986. Alabama Army Ammunition Plant Feasibility Study. Draft Final Report, Report No. DAAK11-83-D-0007. Prepared for U. S. Army Toxic and Hazardous Materials Agency, Aberdeen Proving Ground, MD.

Freeze, R. A. and J. A. Cherry. 1979. Groundwater. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Hostetler, C. J. and R. L. Erikson. 1989. FASTCHEM^{TM} Package. Volume 5: User's Guide to the EICM Coupled Geohydrochemical Transport Code. EPRI EA-5870-CCM, Electric Power Research Institute, Palo Alto, California.

Karickhoff, S. W., D. S. Brown, and T. A. Scott. 1979. Sorption of hydrophobic pollutants on natural sediments. Water Res. 13, 241.

Lyman, W. J., W. R. Reehl and D. H. Rosenblatt. 1982. Handbook of Chemical Property Estimation Methods: Environmental Behavior of Organic Compounds. McGraw-Hill Book Company.

Ogata, A. 1970. Theory of Dispersion in a Granular Medium. U.S. Geological Survey Professional Paper 411-I, U.S. Geological Survey, Reston, Virginia.

Pennington, J. C. and W. H. Patrick, Jr. 1990. Adsorption and Desorption of 2,4,6-Trinitrotoluene by Soils. Journal of Environmental Quality, vol. 19, pp. 559-567.

Schramke, J. A., C. J. Hostetler, and R. L. Erikson. 1994. User's Guide to CTM and PRESCRN1. PNL-9402. Pacific Northwest Laboratory, Richland Washington. 55pp.

Small, M. J. 1984. The Preliminary Pollutant Limit Value Approach: Procedures and Data Base. Technical Report 8210. U. S. Army Bioengineering Research and Development Laboratory (USAMBRDL), Fort Detrick, Frederick, MD.

Smith, J. H., W. R. Mabey, N. Bohonos, B. R. Holt, S. S. Lee, T. W. Chou, D. C. Bomberger, and T. Mill. 1977. Environmental Pathways of Selected Chemicals in Freshwater Systems, Part I. Background and Experimental Procedures. SRI International, Menlo Park, CA. Final Report, EPA Contract 68-03-2227.

Spanggord, R. J., T. Mill, T. W. Chou, W. R. Mabey, J. H. Smith, and S. Lee. 1980a. Environmental Fate Studies on Certain Munition Wastewater Constituents. Final Report, Phase I - Literature Review. SRI International, Menlo Park, CA.

Spanggord, R. J., T. Mill, T. W. Chou, W. R. Mabey, J. H. Smith and S. Lee. 1980b. Environmental Fate Studies on Certain Munition Wastewater Constituents. Final Report, Phase II - Laboratory Studies. SRI International, Menlo Park, CA.

Wilhelm, R. 1991. Soil Characterization Alabama Army Ammunition Plant (AAAP) Chemical Remediation TNT. U. S. EPA Memo to J. Barksdale.

Wolfe, N. L., T. Y. Ou, and L. Carreira. In Prep. Biochemical Remediation of TNT-Contaminated Soils. Final Report to U. S. Army Corps of Engineers, Waterways Experimental Station, Vicksburg, MS.

Table 1. TNT Concentrations from Surveys at the AAAP (ESE, 1986).

Study Area	Environmental Medium	Explor. Survey	Confirm. Survey	RI Survey
Old Burning Ground	Soils	0.656 μg/g		0.386 μg/g
Sanitary Landfill and Lead Facility				
Red Water Storage Basin	Soils	1.17 μg/g		
	Sediments	0.14 μg/g		
Southern & Northern TNT Manufacturing Areas	Soils	698 μg/g	7,660 μg/g	
	Groundwater	10,270 μg/L	22,000 μg/L	1,910 μg/L
	Sediment	0.258 μg/g		
Aniline Sludge Basin				
Tetryl Manufacturing Area	Sediments	0.077 μg/g		
Flashing Ground & Lead Remelt Facility	Soils	2,350 μg/g		
	Groundwater	0.085 μg/L		
	Surface water	13.9 μg/L		
Red Water Ditch	Soil	3,000 μg/g		22,200 μg/g
Storage Battery/Demolition Debris	Soil		0.453 μg/g	
Crossover Ditch	Soil		0.34 μg/g	
Beaver Pond Drainage System	Surface water	140 μg/L	54.6 μg/L	

Table 2.   Properties of Soils from AAAP (Wilhelm, 1991).

Sample	TNT (ppm)	pH in H2O	% Carbon
SO-1	2065	4.95	1.4
SO-2	6000	4.85	0.8
SO-3	36	4.95	0.2
SO-4	2933	4.7	0.3
SO-5	49	5.05	2.5
SO-6	ND	4.8	8.0
SO-7	0.3	4.75	10.3
SO-8	ND	--	--

ND = Not Detected

Table 3.   Size of TNT Nuggets in Dissolution Experiments.

Sample	Volume (cm^3)	Surface Area (cm^2)	Weight (g)
A	0.270	2.58	0.2037
B	0.700	4.80	0.7344
C	1.540	8.08	1.3187
D	6.600	21.40	4.1991

FIGURE CAPTIONS

Figure 1. Solubilization of TNT. TNT nuggets of various sizes were placed in 1 L of water and their dissolution rates were monitored. Descriptions of the TNT nuggets in Bottles A, B, C, and D are provided in Table 3. Figure provided by L. Carreira, Technology Applications Inc.

Figure 2. The Proposed Mechanism of TNT Reduction with Sediment Extracted Enzyme and Aquatic Weed Stonewort. Figure provided by T. Y. Ou, Technology Applications Inc.

Figure 3. Vertical Cross Section through Flooded Basin. The model calculations assume a saturated soil column underlying surface water and stonewort. It is assumed that no TNT can diffuse through the lower boundary of the soil column and that all TNT that diffuses into the surface water is immediately removed by the stonewort enzyme. The laboratory experiments were performed using a 2-cm thick layer of soil.

Figure 4. Diffusion Model. The soil column is divided into layers. Diffusion from layer A into the surrounding layers is described by a seven-layer probability vector that gives the probability that the mass in layer A will move into the surrounding layers during one time step.

Figure 5. Case 1: TNT Diffusion through Soil Column. The initial concentration of TNT is equal to the solubility of TNT [5.7236×10^{-4} M (130 mg/L)].

Figure 6. Case 2: TNT Dissolution and Diffusion. A. TNT Concentrations in Solution. The maximum TNT concentration is 5.7236×10^{-4} M (130 mg/L). The concentration "steps" observed are associated with the loss of solid phase TNT in each layer of the model. B. Solid Mass of TNT in Each Layer. The mass of one mole of TNT is 227 gms; the mass of 5×10^{-8} moles of TNT is 1.14×10^{-2} mg.

Figure 7. Case 3: TNT Adsorption and Diffusion. The initial TNT concentration in solution is 4.4×10^{-5} M or 10 mg/L TNT.

Figure 8. Case 4: TNT Dissolution, Adsorption and Diffusion. The adsorption process dampens the concentration "steps" observed in each layer in Case 2. The initial concentration of TNT is 5.7236×10^{-4} M or 130 mg/L.

Figure 9. Comparison between Analytical and Numerical Solutions. A. Analytical solution from Ogata (1970). B. Numerical solution using diffusion algorithm.

SOLUBILIZATION OF TNT
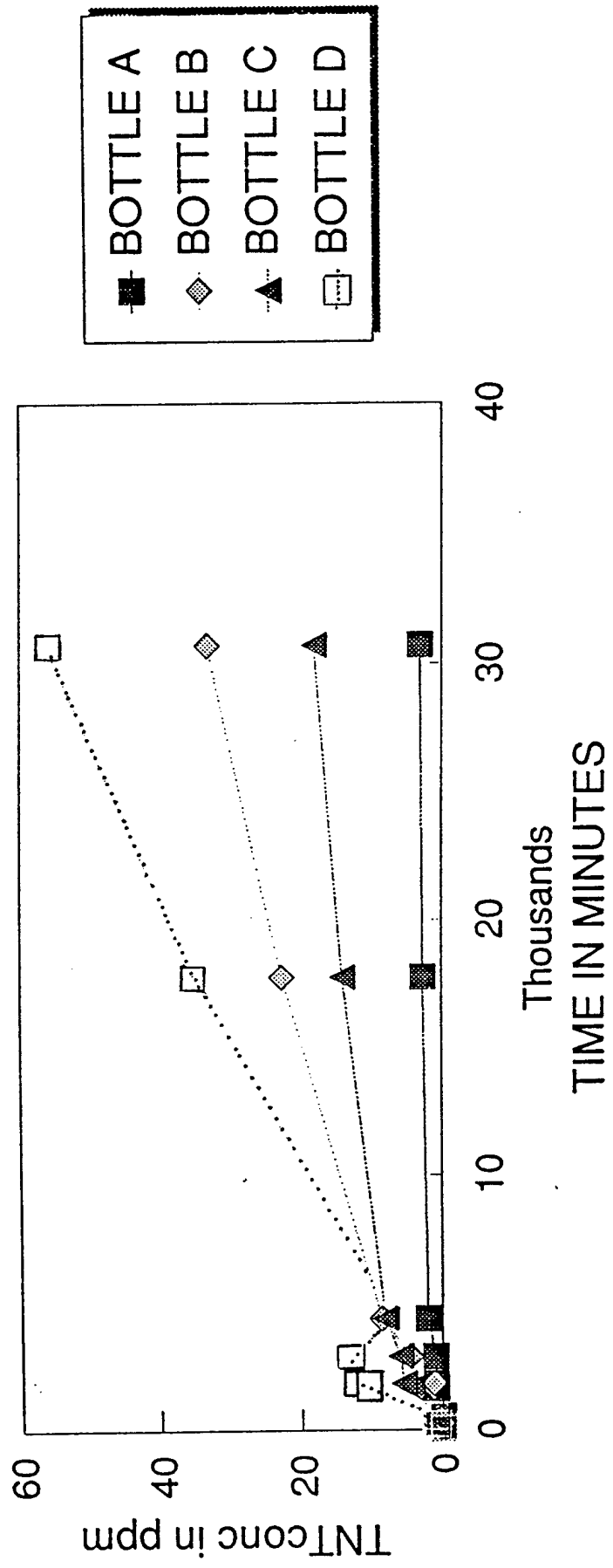
NUGGETS OF TNT IN ONE LITER BOTTLES

Figure 1

The Proposed Mechanism of TNT Reduction with Sediment Extracted Enzyme and Aquatic Weed Stonewort
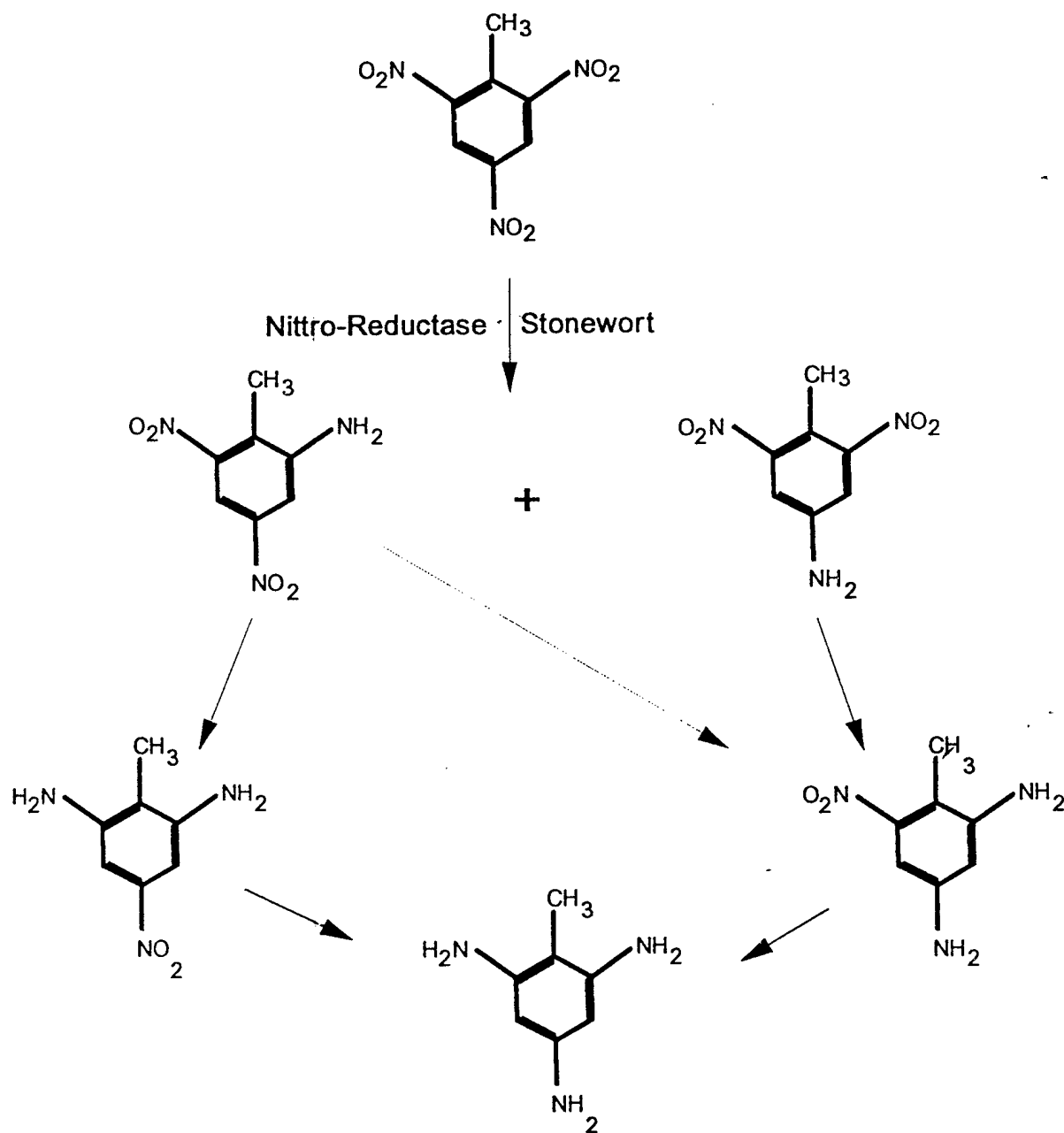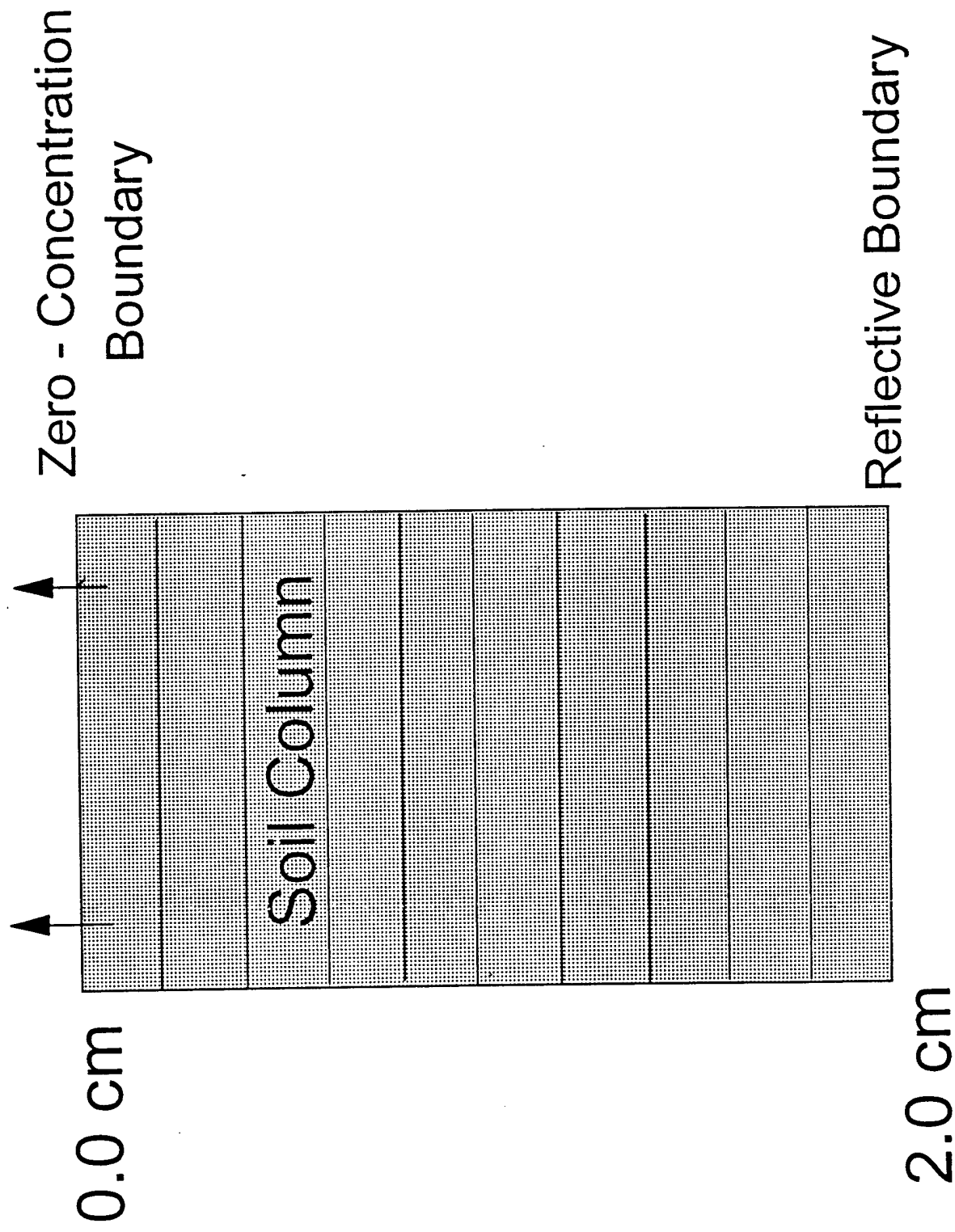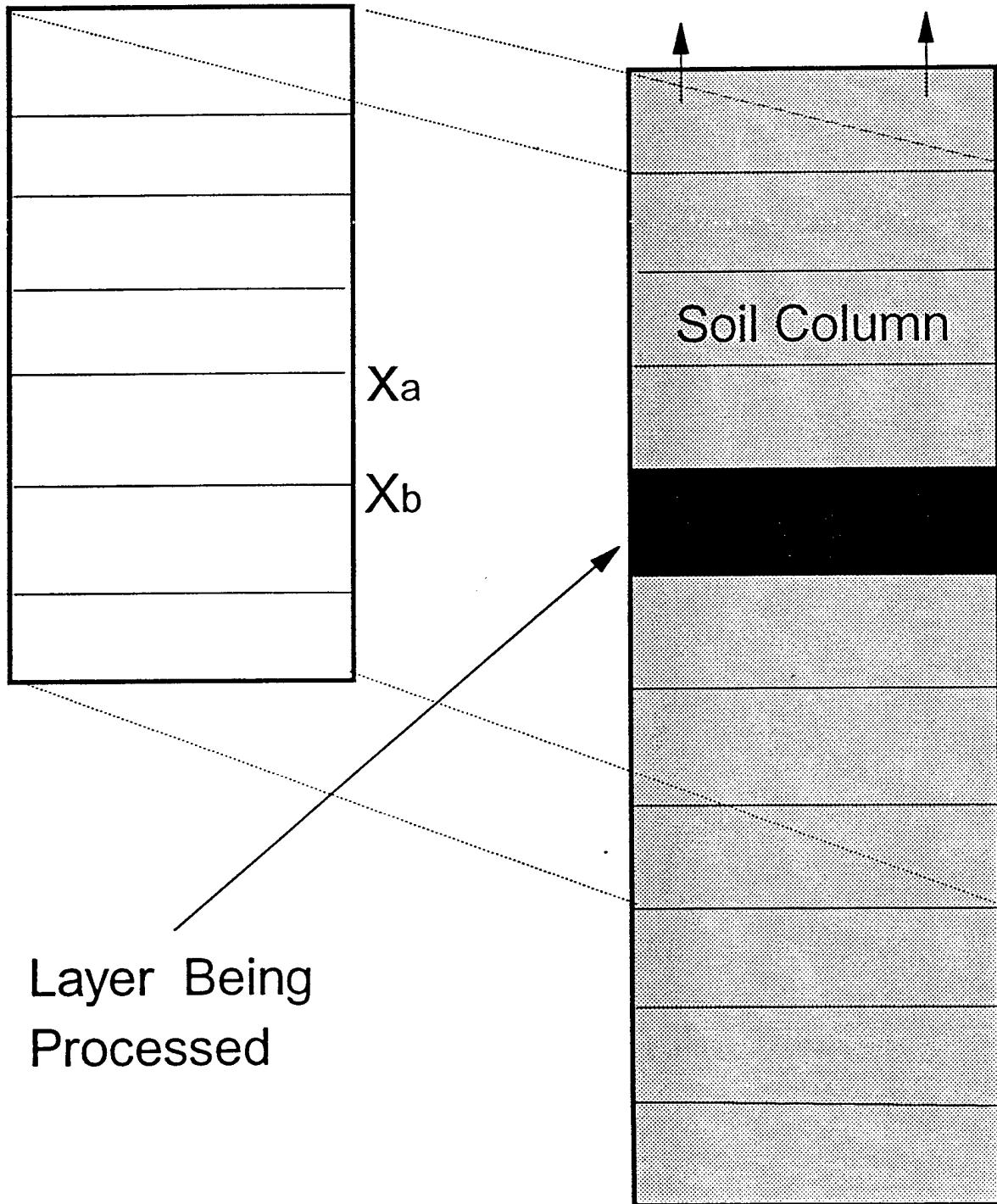

Figure 2

Surface Water and Plants

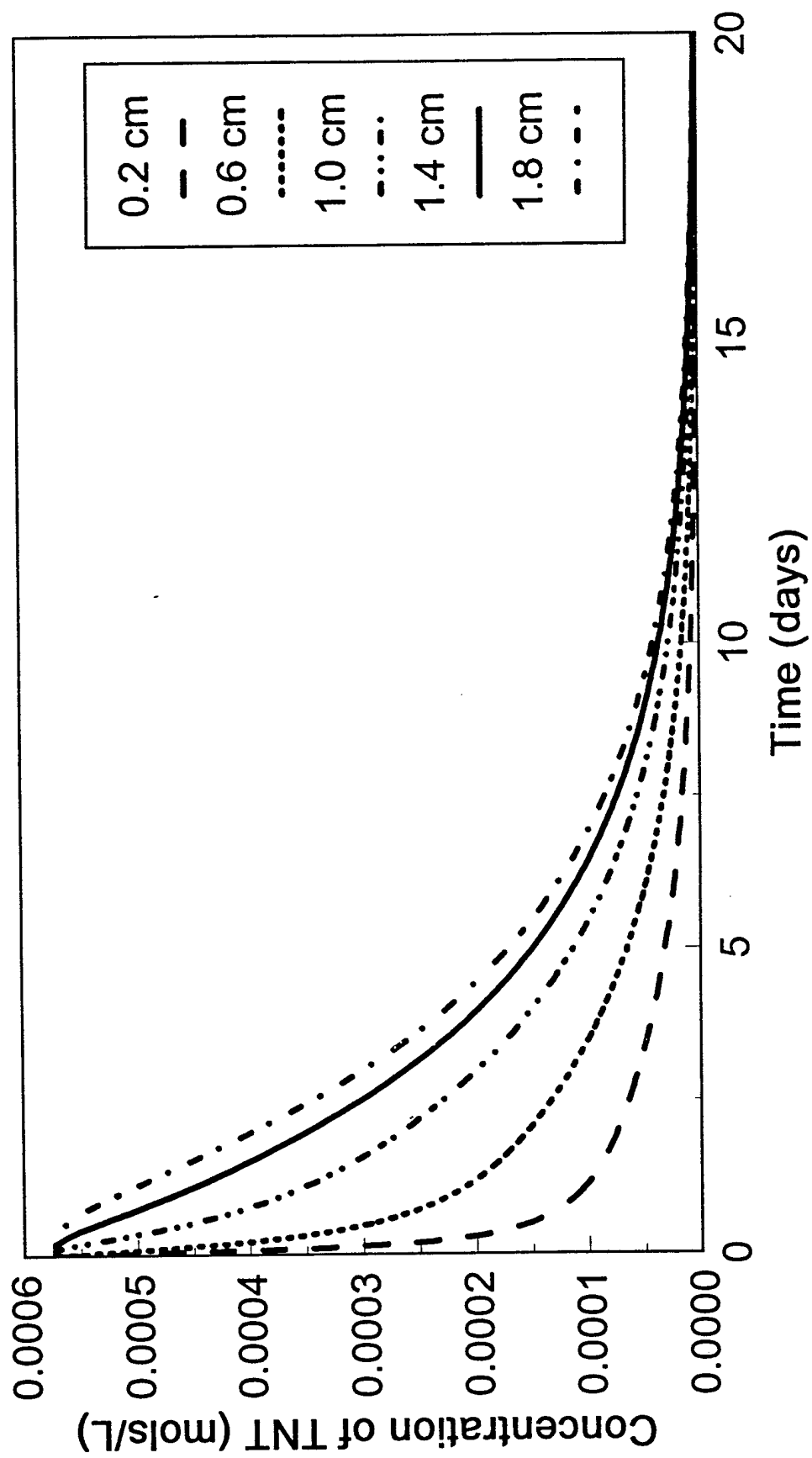Zero - Concentration Boundary

Reflective Boundary
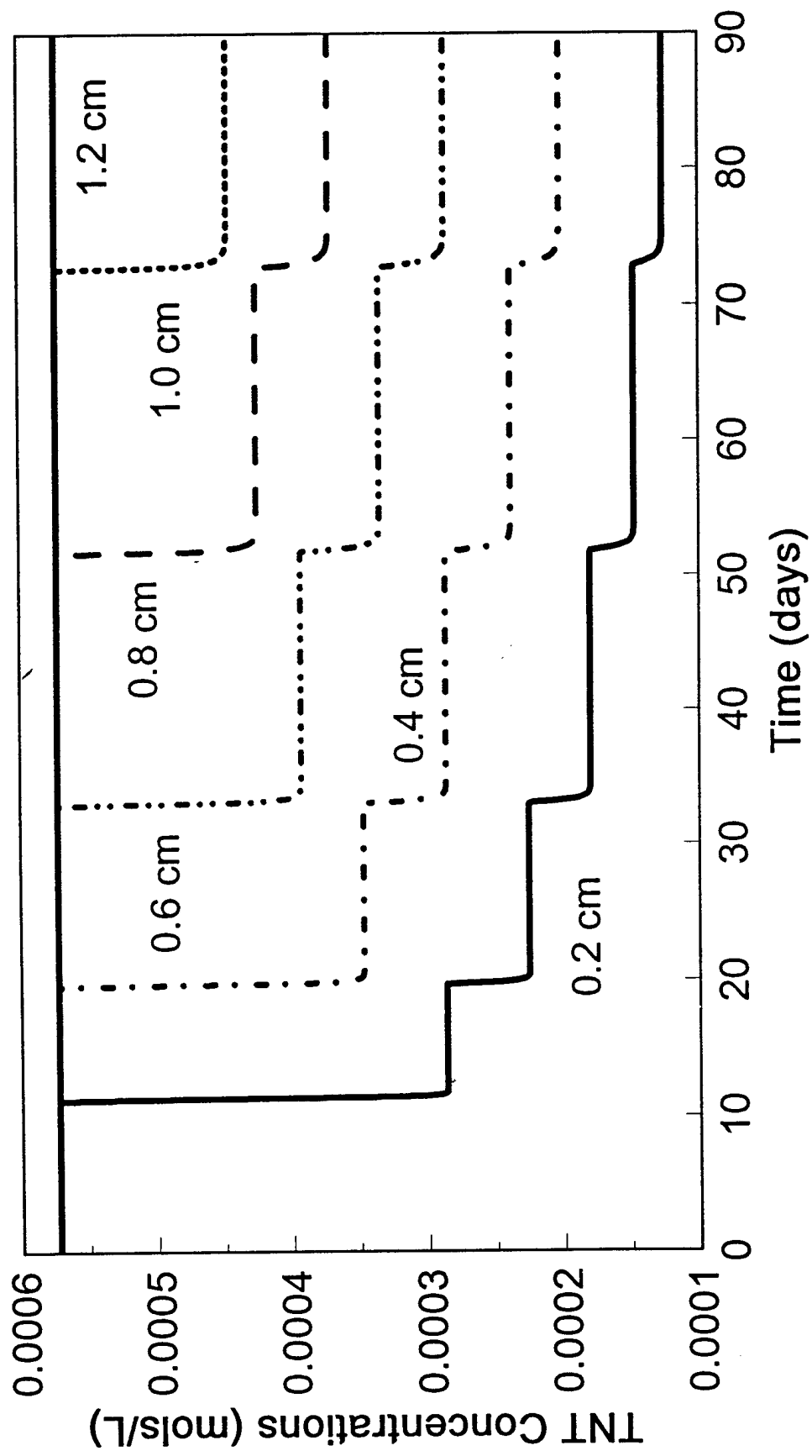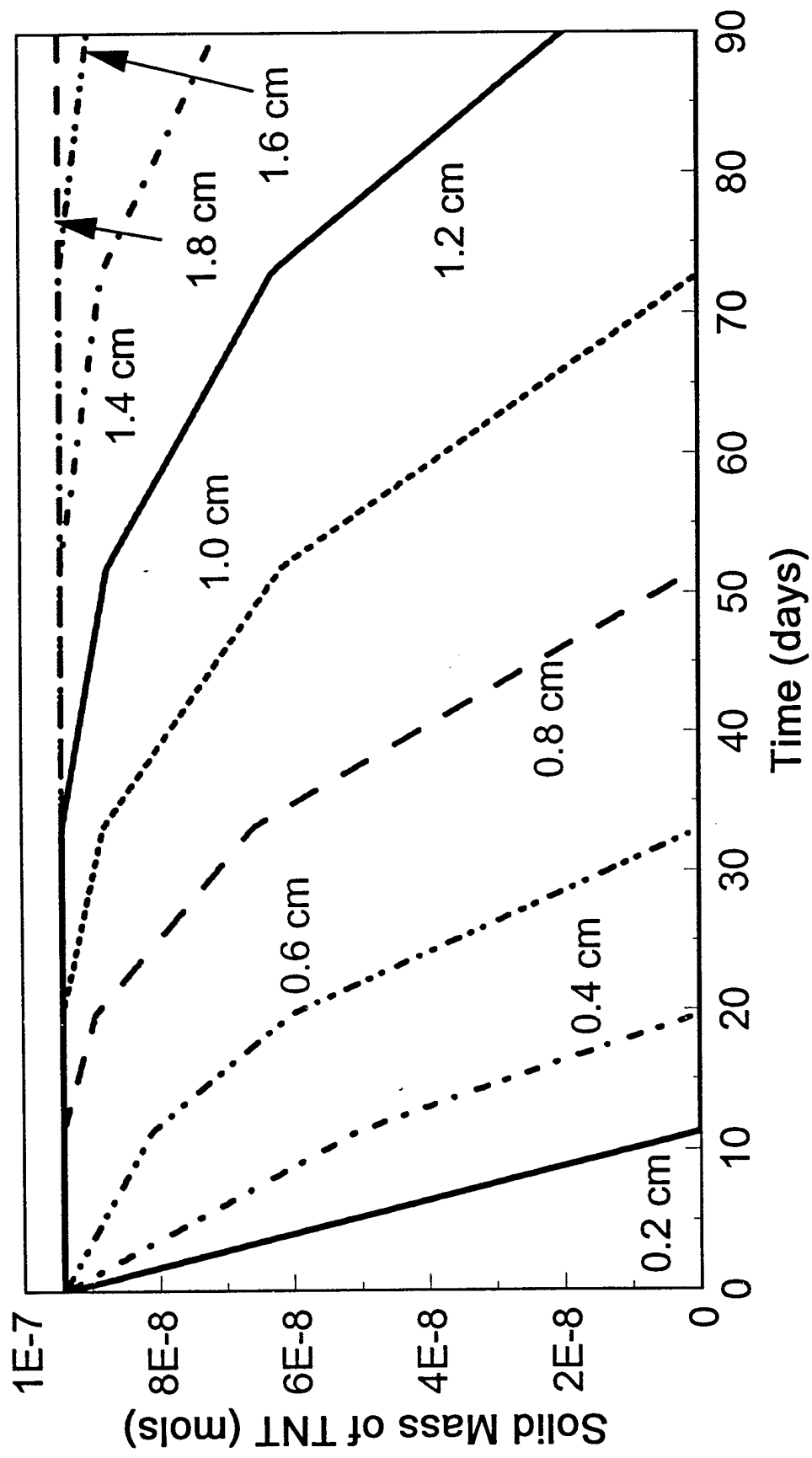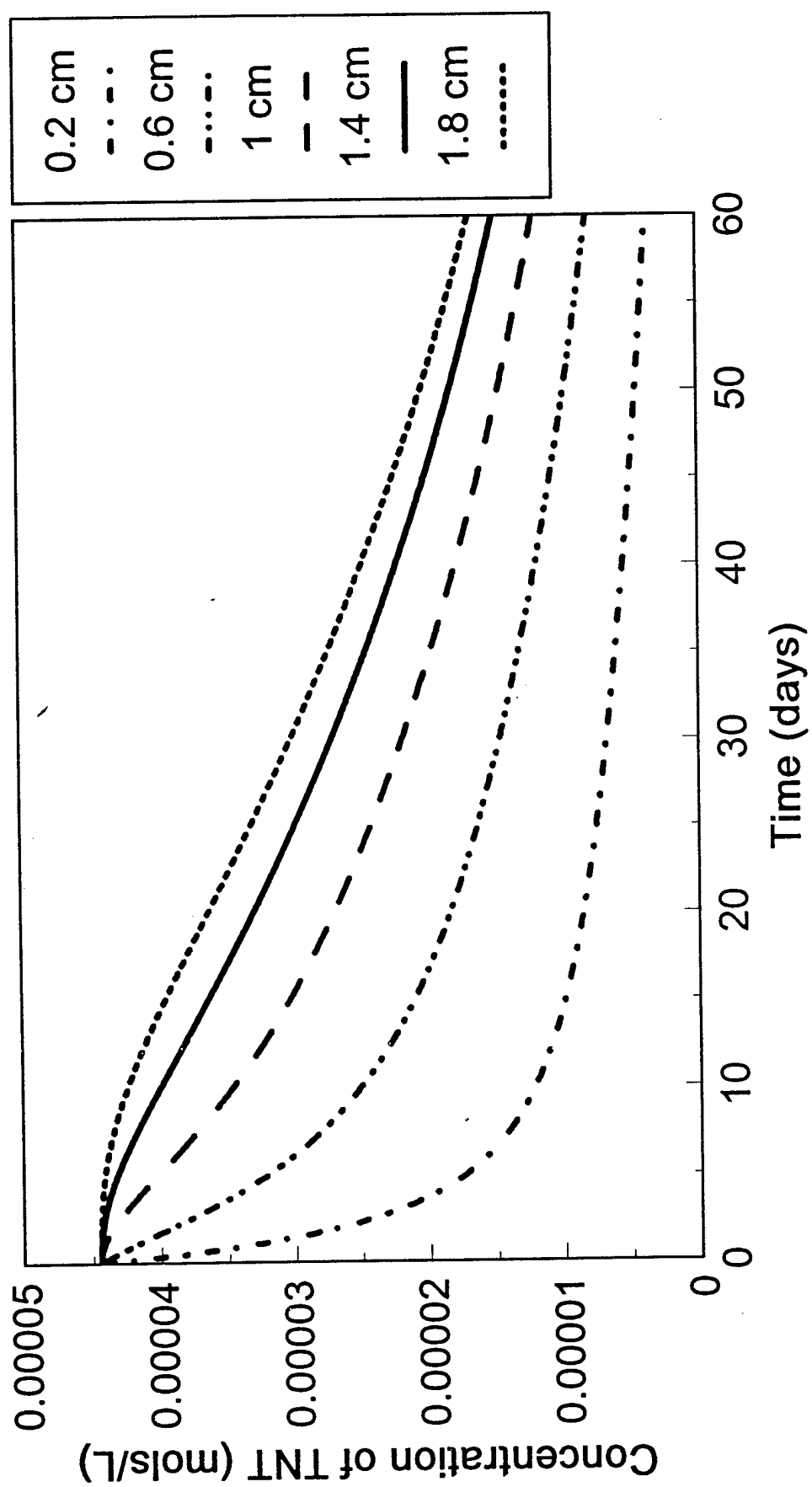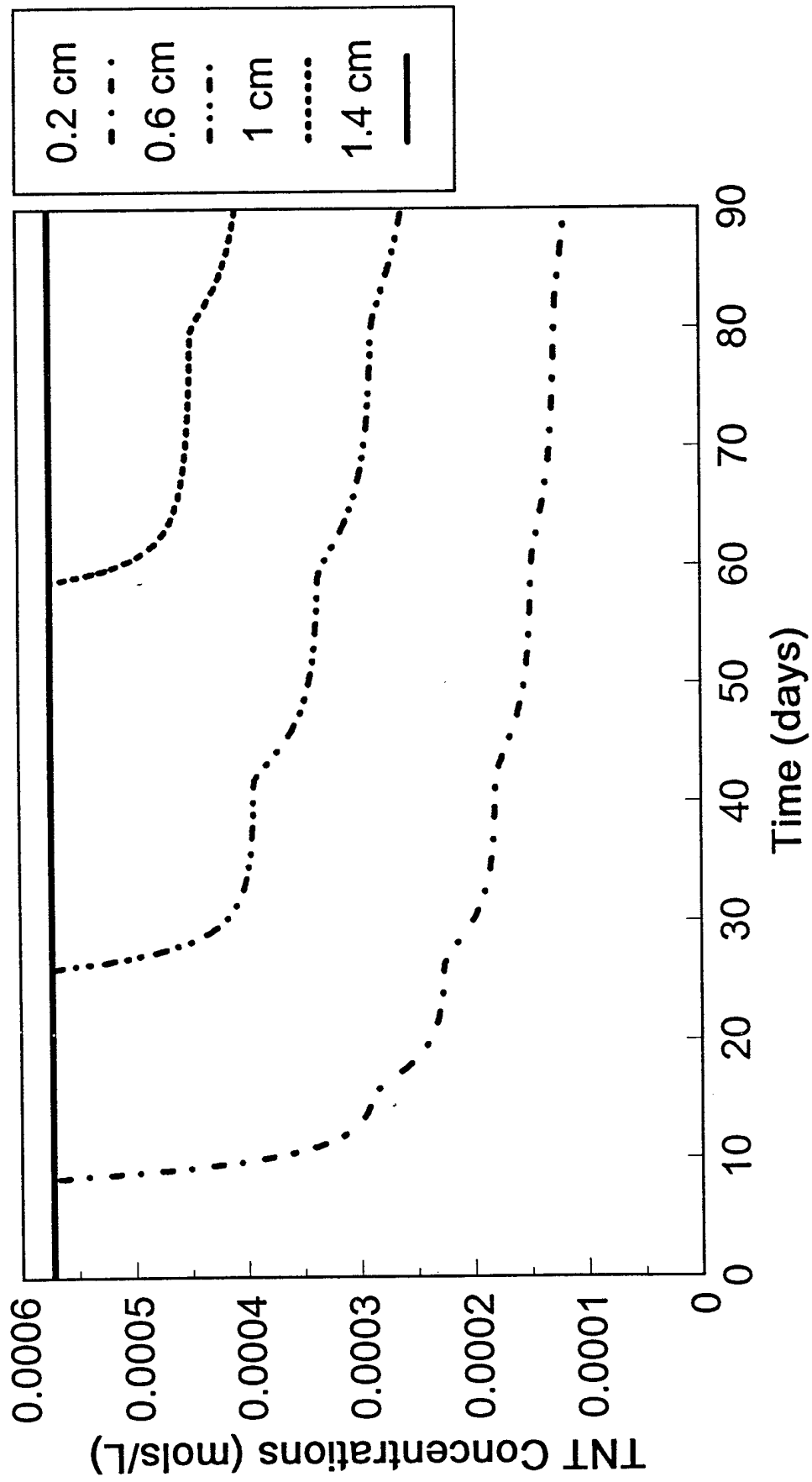
Soil Column

0.0 cm

2.0 cm

Figure 3

Figure 4

Figure 5

Figure 6A

Figure 6B

Figure 7

Figure 8

Figure 9A

Figure 9B